SUPPLEMENTARY MATERAILS

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5 **Supplementary Table S1.** Target, primer sequences and amplification conditions for qPCR assays used in this study

Assays	Target	Primer or probe sequence (5´-3´)[a]	Amplification conditions	References
E. coli (EC)	23S rRNA	F: GGT AGA GCA CTG TTT TGG CA R: TGT CTC CCG TGA TAA CTT TCTC P: FAM-TCA TCC CGA CTT ACC AAC CCG-TAMRA	10 min at 95 °C, 40 cycles of 15 s at 95 °C, 60 s at 60 °C	(2)
Enterococcus spp. (ENT)	23S rRNA	F: AGA AAT TCC AAA CGA ACT TTG R: CAG TGC TCT ACC TCC ATC ATT P: FAM-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-TAMRA	10 min at 95 °C, 40 cycles of 15 s at 95 °C, 2 min at 60 °C	(3)
Bacteroides HF183	16S rRNA	F: ATC ATG AGT TCA CAT GTC CCG R: TAC CCC GCC TAC TAT CTA ATG	10 min at 95 °C, 40 cycles of 30 s at 95 °C, 60 s at 53 °C, 60 s at 60 °C	(1, 6)
Human adenoviruses (HAdVs)	Hexon gene	F: GCC ACG GTG GGG TTT CTA AAC TT R: GCC CCA GTG GTC TTA CAT GCA CAT C P: TGC ACC AGA CCC GGG CTC AGG TAC TCC GA	10 min at 95°C, 40 cycles of 15 s at 95°C, 20 s at 60 °C and 20 S at 95°C	(4)
Human polyomaviruses (HPyVs)	Homologous T antigen	F: AGT CTT TAG GGT CTT CTA CCT TT R: GGT GCC AAC CTA TGG AAC AG P: FAM-TCA TCA CTG GCA AAC AT-MGBNFQ	10 min at 95°C, 40 cycles of 15 s at 95°C, 15 s at 55°C and 60 s 60°C	(5)

6 [a] F: forward primer; R: reverse primer; P: probe; FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine
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Supplementary Table S2: Performance characteristics (range) of *Escherichia coli* (EC), *Enterococcus* spp.
(ENT), *Bacteroides* HF183 (HF183), human adenoviruses (HAdVs) and human polyomaviruses (HPyVs) qPCR
assays

qPCR assays	Slope	Amplification efficiencies (E)	Correlation coefficient (r^2)
EC	–3.362 to –3.414	96.3% to 98.6%	0.997 to 1.000
ENT	–3.231 to –3.393	97.1% to 104%	0.993 to 0.999
HF183	–3.256 to –3.279	101% to 103%	0.999 to 1.000
HAdVs	–3.240 to –3.435	95.5% to 103%	0.993 to 0.997
HPyVs	–3.434 to –3.461	94.5% to 94.9%	0.995 to 0.988

E: Amplification efficiency = $10^{(1/-\text{slope})}/2$.

r^2: denotes the correlation coefficient of determination representing the proportion of variability accounted for by
the linear model.

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Supplementary Table S3: *P* values of Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post-test among fecal indicator bacteria (FIB) and human fecal markers (HFMs) within each wastewater treatment plant (WWTP)

|	EC	ENT	HF183	HAdVs
WWTP A				
ENT	< 0.0001			
HF183	0.0002	0.1959		
HAdVs	< 0.0001	< 0.0001	< 0.0001	
HPyVs	< 0.0001	< 0.0001	< 0.0001	0.0060
WWTP A				
ENT	< 0.0001			
HF183	0.5438	< 0.0001		
HAdVs	< 0.0001	< 0.0001	< 0.0001	
HPyVs	< 0.0001	< 0.0001	< 0.0001	> 0.9999
WWTP A				
ENT	0.0090			
HF183	> 0.9999	0.2335		
HAdVs	< 0.0001	< 0.0001	< 0.0001	
HPyVs	< 0.0001	< 0.0001	< 0.0001	> 0.9999
Pooled datasets[a]				
ENT	< 0.0001			
HF183	0.1629	< 0.0001		
HAdVs	< 0.0001	< 0.0001	< 0.0001	
HPyVs	< 0.0001	< 0.0001	< 0.0001	0.1269

EC: *E. coli*
ENT: *Enterococcus* spp.
HAdVs: Human adenoviruses
HPyVs: Human polyomaviruses
[a]:data from three WWTPs were pooled.

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69 **Supplementary Table S4:** Spearman correlation (*r*) matrix among fecal indicator bacteria (FIB) and human fecal
70 markers (HFMs) within each wastewater treatment plant (WWTP). *P* values are shown in the brackets.

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	EC	ENT	HF183	HAdVs
WWTP A				
ENT	0.728 (*P* < 0.0001)			
HF183	0.696 (*P* < 0.0001)	0.862 (*P* < 0.0001)		
HAdVs	0.329 (*P* < 0.001)	0.468 (*P* < 0.001)	0.670 (*P* < 0.0001)	
HPyVs	-0.023 (*P* = 0.820)	0.036 (*P* = 0.722)	0.329 (*P* < 0.001)	0.466 (*P* < 0.001)
WWTP A				
ENT	0.606 (*P* < 0.0001)			
HF183	0.950 (*P* < 0.0001)	0.549 (*P* < 0.0001)		
HAdVs	0.435 (*P* < 0.001)	0.885 (*P* < 0.0001)	0.383 (*P* < 0.001)	
HPyVs	0.308 (*P* < 0.001)	0.486 (*P* < 0.0001)	0.207 (*P* < 0.05)	0.420 (*P* < 0.0001)
WWTP A				
ENT	0.531 (P < 0.0001)			
HF183	0.890 (P < 0.0001)	0.562 (P < 0.0001)		
HAdVs	0.427 (P < 0.0001)	0.235 (P < 0.05)	0.420 (P < 0.0001)	
HPyVs	-0.039 (0.702)	-0.089 (*P* = 0.379)	0.048 (*P* = 0.636)	0.300 (*P* < 0.001)
Pooled datasets[a]				
ENT	0.259 (*P* < 0.001)			
HF183	0.845 (*P* < 0.0001)	0.338 (*P* < 0.0001)		
HAdVs	0.413 (*P* < 0.0001)	0.515 (*P* < 0.0001)	0.416 (*P* < 0.0001)	
HPyVs	0.189 (*P* < 0.05)	0.185 (*P* < 0.05)	0.171 (*P* < 0.05)	0.448 (*P* < 0.0001)

72 EC: *E. coli*
73 ENT: *Enterococcus* spp.
74 HAdVs: Human adenoviruses
75 HPyVs: Human polyomaviruses
76 [a]:data from three WWTPs were pooled.
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Supplementary Table S5: Concentrations (gene copies per mL) of sewage-associated *Bacteroides* (HF183), human adenoviruses (HAdVs), and human polyomaviruses (HPyVs) in raw wastewater samples collected from three wastewater treatment plants (WWTPs) (A, B and C) in Australia.

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Wastewater treatment plants	Sampling events	HF183	HAdVs	HPyVs
WWTP A	1	1.58×10^6	8.40×10^3	3.80×10^3
	2	1.86×10^6	1.41×10^4	7.46×10^3
	3	1.51×10^6	2.48×10^4	6.59×10^3
	4	2.18×10^6	5.07×10^4	1.11×10^4
	5	1.04×10^6	1.39×10^4	6.90×10^3
	6	5.76×10^5	7.07×10^3	5.88×10^3
	7	8.34×10^5	1.69×10^4	3.70×10^3
	8	2.41×10^6	2.37×10^4	7.12×10^3
	9	1.25×10^6	4.16×10^4	9.81×10^3
	10	6.28×10^4	1.70×10^4	9.27×10^3
	11	4.93×10^5	9.96×10^3	6.59×10^3
WWTP B	1	1.44×10^6	7.15×10^3	5.90×10^3
	2	1.56×10^6	7.67×10^3	4.95×10^3
	3	1.69×10^6	9.73×10^3	5.76×10^3
	4	4.03×10^5	5.66×10^3	5.51×10^3
	5	2.75×10^4	1.11×10^4	7.44×10^3
	6	1.18×10^5	3.49×10^3	6.88×10^3
	7	9.04×10^5	2.40×10^3	7.60×10^3
	8	1.06×10^6	6.94×10^3	3.96×10^3
	9	4.05×10^5	2.50×10^3	4.04×10^3
	10	2.99×10^5	2.11×10^3	2.62×10^3
	11	7.30×10^5	3.42×10^3	5.61×10^3
WWTP C	1	1.78×10^5	1.53×10^4	3.61×10^3
	2	5.47×10^4	2.97×10^3	4.34×10^3
	3	4.19×10^5	1.71×10^4	8.05×10^3
	4	3.08×10^5	2.31×10^4	5.77×10^3
	5	2.43×10^5	3.92×10^3	5.07×10^3
	6	6.58×10^5	4.91×10^3	5.59×10^3
	7	1.04×10^5	7.25×10^3	3.55×10^3
	8	1.05×10^5	1.86×10^4	6.10×10^3
	9	8.03×10^4	2.72×10^3	5.41×10^3
	10	4.10×10^4	1.53×10^4	1.39×10^4
	11	8.17×10^4	2.93×10^3	4.97×10^3

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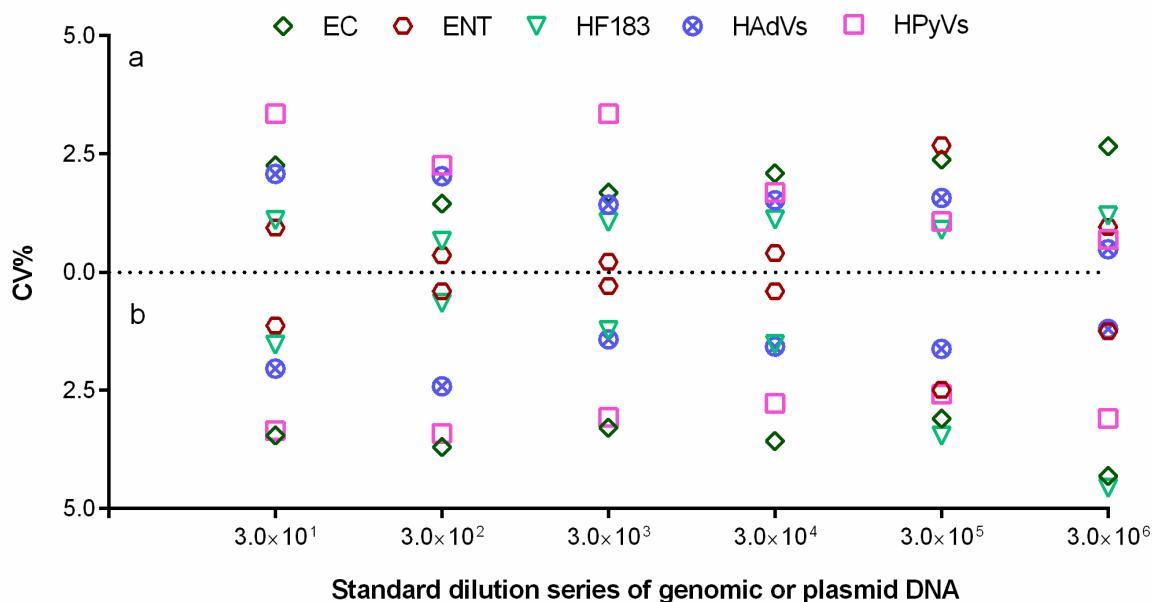
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108 **Supplementary Fig. S1:** The mean (a) intra-assay repeatability and (b) inter-assay reproducibility coefficient of
109 variation (CV) for the EC (*E. coli*), ENT (*Enterococcus* spp.), sewage-associated *Bacteroides* HF183 (HF183),
110 HAdVs (human adenoviruses) and HPyVs (human polyomaviruses) qPCR assays within the range of 3.0×10^1 to
111 3.0×10^6 standard series

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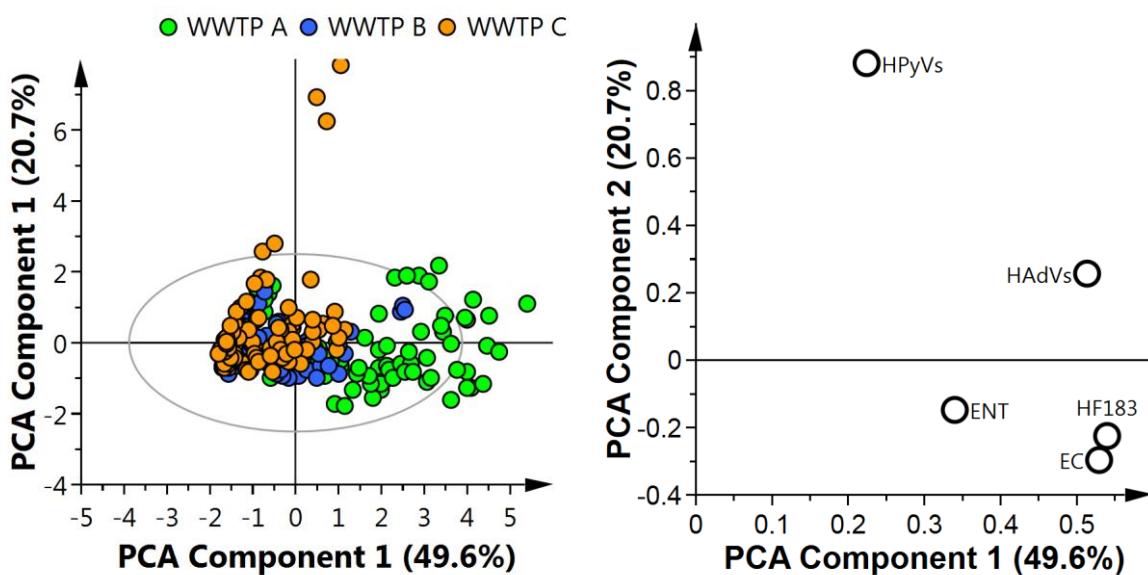
Supplementary Fig. S2: Principal Component Analysis (PCA) of the concentrations (gene copies per mL) of *Escherichia coli* (EC), *Enterococcus* spp. (ENT), *Bacteroides* HF183 (HF183), human adenoviruses (HAdVs), and human polyomaviruses (HPyVs) in raw wastewater samples collected from three WWTPs (A, B and C) in Australia. Figure (a) represents the PCA score scattered plot and Figure (b) represents PCA loading scatter plot.

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Supplementary Fig. S3: Distance of observation plot of the PCA data

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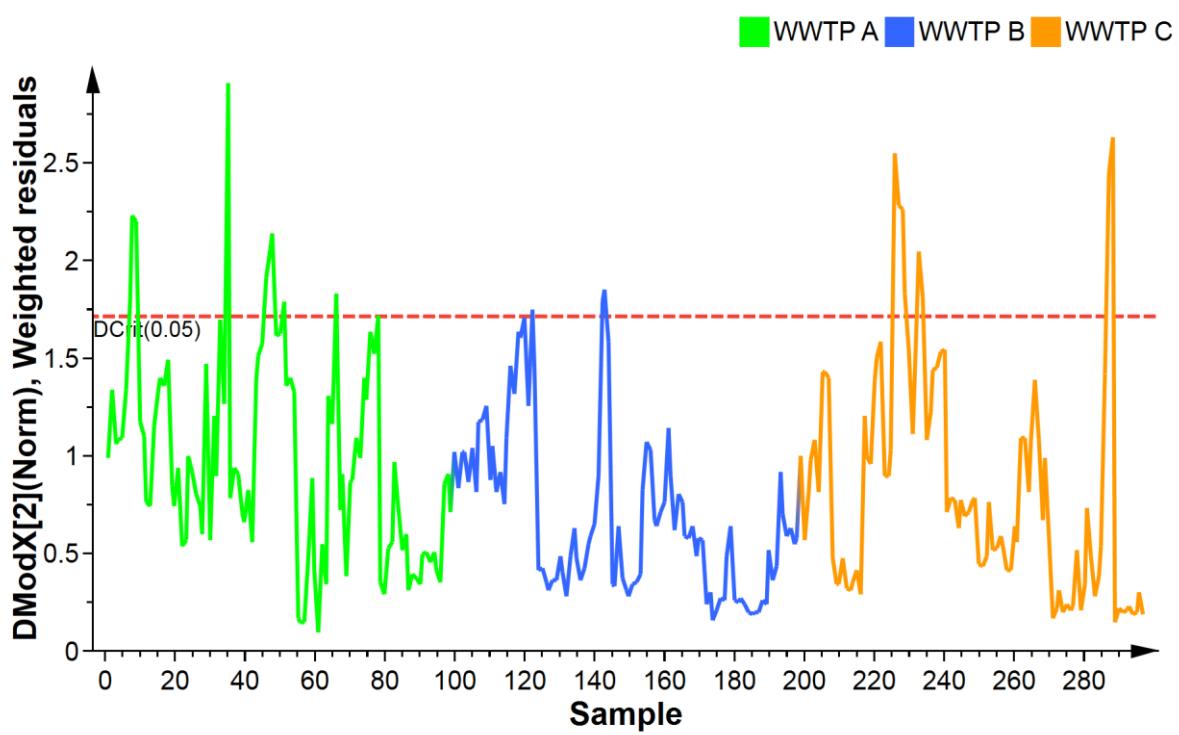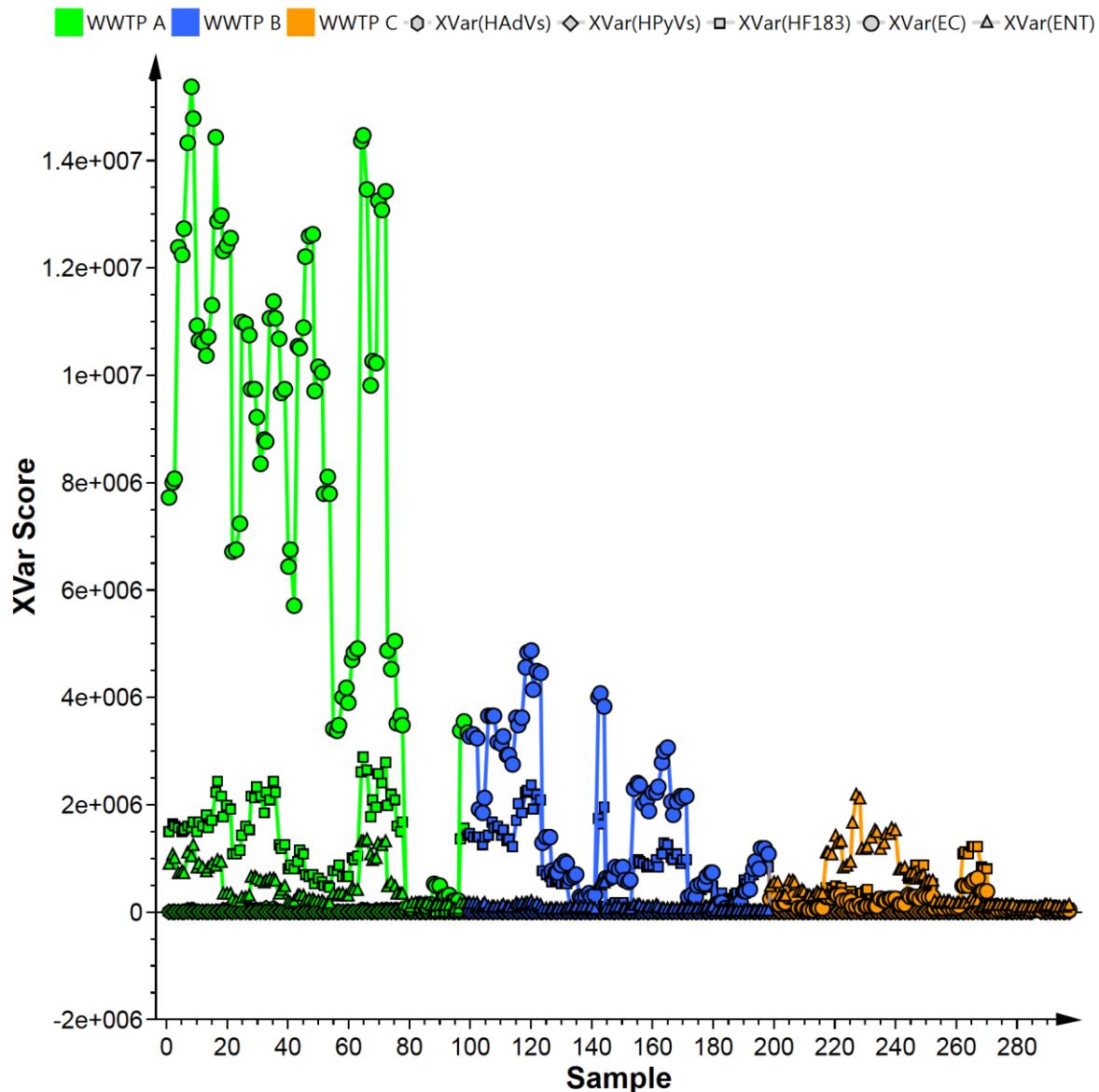
Supplementary Fig. S4: Variable contribution plot for concentrations (gene copies per mL) of *Escherichia coli* (EC), *Enterococcus* spp. (ENT), *Bacteroides* HF183 (HF183), human adenoviruses (HAdVs), and human polyomaviruses (HPyVs) across the three WWTPs (A, B and C)

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